Acta vet. scand. 1986, 27, 91—102.

From the Department of Food Hygiene, College of Veterinary Medicine, Helsinki, Department of Medical Microbiology, University of Turku, Turku and Department of Serology and Bacteriology, University of Helsinki, Helsinki, Finland.

SPORADIC CRYPTOSPORIDIOSIS IN A RURAL POPULATION IS ASYMPTOMATIC AND ASSOCIATED WITH CONTACT TO CATTLE

By

Suvi Pohjola, Anssi M. M. Jokipii and *Liisa Jokipii*

POHJOLA, SUVI, ANSSI M. M. JOKIPII and LIISA JOKIPII: *Sporadic cryptosporidiosis in a rural population is asympomatic associated with contact to cattle.* Acta vet. scand. 1986, 27, 91—102. — Fecal samples each followed by a completed questionnaire were obtained from 233 persons representing 80 households. Using the formalin-ether concentration method combined with the acid-fast staining oocysts of Cryptosporidium were found in 7 (3 %) persons. 63 persons had contact to cattle, and asymptomatic cryptosporidiosis was diagnosed in 6 of them. Cryptosporidiosis was not found in any of the 51 persons with contact to some other animal but not cattle, and none of the 7 Cryptosporidium positive persons had been traveling abroad. We also obtained fecal samples from 15 calves from 7 households. Six of the calves (40 %) harbored cryptosporidia and manifest enteritis was detected in those under age of 1 month. Five out of 14 farms had problems of neonatal calf diarrhea. Human cryptosporidiosis was more frequent on such problem farms. The results indicate that in rural population, human cryptosporidiosis is mainly asymptomatic and occurs mainly in residents of cattle-rearing farms.

animal contacts; zoonosis.

Genus Cryptosporidium, a coccidian protozoan was first described in histological sections of the gastric glands of the common mouse by *Tyzzer* (1907). Since the 1970's cryptosporidia have been identified worldwide in gastrointestinal and/or respiratory tract of all vertebrate classes (*Tzipori* 1983 a). Cross transmission experiments have been carried out between several mammalian species and they show no host specificity (*Tzipori* 1983 a). At present 4 named species of Cryptosporidium, one for each vertebrate class, are considered to exist (*Levine* 1984).

Since the 1970's many individual case reports and large outbreaks of neonatal calf diarrhea attributed to Cryptosporidium have been published (*Tzipori et al.* 1980, *Anderson* 1981, *Anderson & Hall* 1982, *Levine* 1984). In humans Cryptosporidium was first sporadically detected in immunocompromised persons as a cause of chronic unremitting diarrhea (*Meisel et al.* 1976, *Lasser et al.* 1979, *Weisburger et al.* 1979, *Sloper et al.* 1982, *Miller et al.* 1982). The first case in Finland in 1981 was a diarrheic calf of 3 weeks of age (*Pohjola* 1983). In a following field survey Cryptosporidium was detected in 53 % of 68 calves from 8 dairy herds (*Pohjola et al.* 1986). The first human cases in Finland were found in 1982 in a 3-month survey, in which cryptosporidial oocysts were detected in 14 out of 1422 (1.0 %) patients with gastroentestinal symptoms (*Jokipii et al.* 1983).

The life cycle of the monoxenous Cryptosporidium (i.e. the parasite requires only 1 host to the entire life cycle) occurs in the brush border of enterocytes (*Tzipori* 1983 a). The prepatent period culminates in shedding of sporulated oocysts, which transmit the infection (*Tzipori* 1983 a, *Levine* 1984). Cryptosporidiosis can be diagnosed be detecting the oocysts from stools (*Tzipori* 1983, *Pohjola et al.* 1986).

The epidemiological knowledge of Cryptosporidium is scanty. The prevalence of infection and the occurrence of carrier state in man and various animals are unknown (*Tzipori* 1983 a). The source of infection in most human cases has remained unrevealed although some cases with zoonotic implications have been published (*Reese et al.* 1982, *Anderson et al.* 1982, *Current et al.* 1983, *Pohjola et al.* in print). According to our earlier experience cryptosporidiosis has been unrelated to animal contacts (*Jokipii et al.* 1983), and this also been the conclusion of British investigators, who find highest prevalences of cryptosporidiosis in children with gastroenteritis (*Hunt et al* 1984, *Casemore & Jackson* 1984). In Finland cryptosporidiosis has been rarely found in children, and we have recently confirmed the lack of association with animal contacts and reported the highest prevalences in young adults and a strong correlation of cryptosporidiosis and recent traveling abroad (*Jokipii et al.* 1985).

The purpose of this study was to evaluate the prevalence of cryptosporidiosis and the occurrence of carrier state among residents of a rural community, and to look for associations of the infection between humans and calves.

MATERIALS AND METHODS

Geographic area, population, time of study

The investigation was carried out at Tohmajärvi, a 668-km^2 community in the province of North Carelia in the eastern part of Finland from June to August 1983. According to the local authorities the population of the community was at that time 6200, of whom 2060 had residence in a household rearing livestock, and 580 had direct daily contact to cattle.

Data and sample collection

The primary subjects of this study were persons visiting the health center for reasons other than acute illness e.g. mother and child care, health certificate. They were informed about the purpose of the study and asked to volunteer with their family members. Three hundred questionnaires and an equal number of formalin containers for fecal samples were distributed by the health care workers. Detailed information was inquired about i) demographic data (age, sex, occupation), ii) occurrence of gastrointestinal symptoms, iii) exposure to animals classified as daily contact, residence in a household keeping animals, or no animal contacts, iv) clinical symptoms of the animals of the household at the time of and for a month prior to the investigation, v) traveling abroad. At the same time the subjects were asked to submit fecal samples from themselves, from their family members and from their calves.

Parasitological methods

The formalin-ether concentration method of *Ritchie's* (1948) as modified by *Allen & Ridley* (1970) was used for fecal samples of both human and animal origin. The sediments were stained with the modified Ziehl-Neelsen technique (*Henriksen & Pohlenz* 1981) as previously described (*Jokipii et al.* 1983, *Pohjola et al.* 1986). The whole stained area was examined under oil immersion by using a × 1000 objective lens.

Statistical methods

The significance of differences between frequencies was subjected to Fisher's exact test, one-tailed (*Zar* 1974).

RESULTS

Humans

Fecal samples and questionnaires were obtained from 233 persons. Cryptosporidium oocysts were found in 7 (3.0 %) of them. The sex distribution was unremarkable: 3 of the 104 males (2.9 %) and 4 of 129 females (3.1 %) harbored cryptosporidia. The mean age of the subjects was 31.7 years (S.D. 24.8) and all age groups were represented. The prevalence of Cryptosporidium infection was 2.2 % in children 15 years or younger and 3.5 % in adults. One child among the 52 under the age of 5 years harbored cryptosporidia (Table 1).

Table 1. Cryptosporidium oocyst shedding and age distribution in 233 persons.

Age (years)	No. of persons examined	No. of persons with Cryptosporidium
<1	16	1
1—10	61	0
11—20	27	2
21—30	37	0
31—40	40	2
41—50	29	2
51—60	12	0
<60	11	0
Total	233	7

During 1 month preceding the date of the fecal sample, 23 persons had experienced gastrointestinal symptoms, 19 of them reported diarrhea and 17 abdominal pain. None of the 7 Cryptosporidium positive persons had any gastrointestinal symptoms.

The frequency of persons harboring cryptosporidia was significantly greater among those with contact to cattle, cats, chickens or swine (Table 2). All the contact cats, chickens and swine, however, were from cattle-rearing farms. In 1 person cryptosporidiosis was found without any history of regular animal contacts. He was a mason who worked outdoors and visited also farms.

Cryptosporidiosis was not found in any of the 51 persons with contact to some animals other than cattle Out of the 63 persons exposed to cattle 59 lived on farms rearing livestock. Twenty-one persons were full-time animal handlers with the

Table 2. Cryptosporidium oocyst shedding in 233 persons as related to animal contacts*.

Animal	No. of persons with contact	No. of persons with Cryptosporidia	P**
Cattle	63	6	0.0018
Goat	4	0	
Swine	24	3	0.0257
Chicken	25	3	0.0288
Dog	64	3	
Cat	48	4	0.0346
Rabbit	9	0	
Guinea pig	4	1	
Fur animal	2	0	
Cage bird	6	0	
Total no with contacts	114	6	0.0531
Total without contacts	119		
Total	233	7	

* A single person could have contacts with more than one species of animal.

** Compared to the rest of the subjects without the respective animal contact: Fisher exact test.

most direct and prolonged daily exposure, they had 38 family members. Cryptosporidia were found in 14.3 % of the animal handlers compared to 2.6 % of the family members (Fig. 1, farms 1—14).

Traveling histories were obtained from all the 233 persons, of whom 11 had visited the Soviet Union, 7 the Scandinavian countries, and a few Germany, the Netherlands and Poland. None of the 7 persons shedding cryptosporidia had been abroad during the preceding 6 months.

Calves

A fecal sample was obtained from 15 calves, of which 6 were found to be infected with Cryptosporidium (Fig. 1, farms 1, 3, 6). Oocysts were detected in 3 of the 7 diarrheic and in 3 of the 8 nondiarrheic calves indicating that shedding of oocysts was not associated with the clinical symptoms. Diarrheic calves were all under the age of 1 month, whereas calves subclinically infected were 2.5 to 4 months of age.

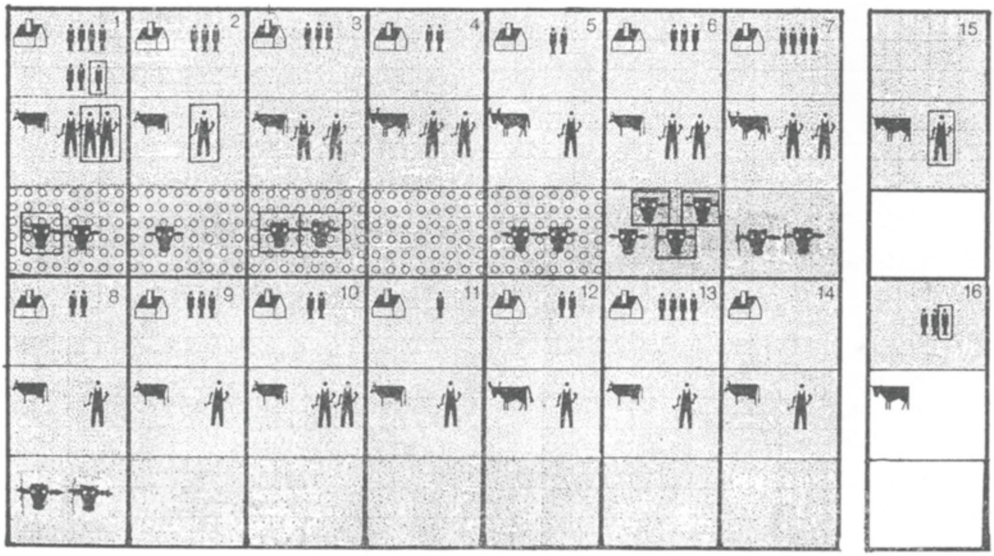

Figure 1. Shedding of Cryptosporidium oocysts in 63 residents of cattle-rearing households.

Out of the 63 persons exposed to cattle, 59 lived in 14 farms (*squares 1—14*) with occupational cattle-rearing. Cryptosporidiosis was diagnosed in 6 of them, 14.3 % of the animal handlers (B) and 2.6 % of their family members (A).

Five out of 14 farms (*squares 1—5*) reported problems of neonatal calf diarrhea (NCD), 15 calves from 7 herds were examined, 6 of the calves (C) (40 %) harbored Cryptosporidium. Human cryptosporidiosis was more frequent on those NCD farms.

Squares 15 and 16: Households with 1 person attending a course on animal husbandry and 3 children staying with their cattle-rearing grandparents.

Household entities

The 233 persons were from 80 different households and the response rate was 89.6 % among all family members of the households. Fifty nine persons on 14 farms (Fig. 1, farms 1—14) and 1 person (Fig. 1, square 15) attending a course on animal husbandry had permanent contact to cattle. The 3 children (Fig. 1, square 16) had contact to cattle only during the summer months, when they stayed with their cattle-rearing grandparents.

There were no statistically significant correlations between Cryptosporidium infections in humans and calves. Five out of 14 farms reported concurrent problems of neonatal calf diarrhea (Fig.1, farms 1—5). Four out of 26 persons excreted oocysts on these farms, whereas no positive cases were found on farms without problems of calf diarrhea. There was a significant differences of occurrence of human cryptosporidiosis between the farms with diarrheal problem (Fig. 1, farms 1—5) compared to the farms without such a problem (Fig. 1, farms 6—14) ($P = 0.03$).

DISCUSSION

In the present study for the first time Cryptosporidium was examined in a restricted population of which every third human had residence on a cattle-rearing farm. This rural population was especially selected for the evaluation of the hypothesis of zoonotic origin of the infection (*Reese et al.* 1982, *Anderson et al.* 1982, *Current et al.* 1983, *Pohjola et al.* 1985 b).

The combination of *Ritchie's* formalin-ether concentration method (1948) as modified by *Allen & Ridley* (1979) and the modified Ziehl-Neelsen technique (*Henriksen & Pohlenz* 1981) were adopted for the diagnostic techniques, since they had been assessed appropriate for organism recovery and identification (*Jokipii et al.* 1983, *Pohjola et al.* 1986 & in print). The organisms retain their morphology and the combination is safe, because oocysts loose their viability in formalin-preserved stools. The sediments will keep unhanged in well stoppered tubes in a refrigerator, and can well be restained after long periods of time. Based on comparative studies on 15 methods the modified Ziehl-Neelsen method on formalin-preserved stools was also recommended by *Garcia et al.* (1983). The formalin-ether concentration technique is not a routine method in veterinary parasitology; in our study it proved to be efficient for Cryptosporidium identification from calf feces.

The prevalence of cryptosporidiosis in all humans in our study was 3.0 %; 9.5 % in persons with contact to cattle and 0.6 % in those without such contacts. The results of our study are evidence against an outbreak of infection and suggest endemicity, since the 7 positive human cases were from 5 households with no apparent connections. In Australia the prevalence of cryptosporidiosis among hospitalised children with gastroenteritis was higher (4.8 %) than in adults (1.6 %) (*Tzipori et al.* 1983 b). In our material children were not more frequently infected than were adults.

None of our subjects with Cryptosporidium reported any symptoms of gastrointestinal infection during the study. Reports of shedding oocysts without symptoms has so far been reported in 2 previously healthy persons taking care of infected calves (*Current et al.* 1983). Intensified survey activities and effective noninvasive screening methods will certainly reveal the prevalence of asymptomatic carrier state in large populations.

Cryptosporidial oocysts were found in 40 % of the calves, and the subclinically infected ones were older than the diarrheic ones. The age-related increase in resistance to diarrhea while shedding oocysts was also noticed in the previous field survey, in which Cryptosporidium was found in 53 % of 68 calves and 72 % of the positive calves contracted their infection within the first week of life (*Pohjola et al.* 1986). The subclinical shedding of oocysts in the present study may have resulted from the later initial challenge or lower infectious pressure, since all the subclinically infected calves were from a farm with no diarrheal problems of calves.

The first human case was reported in 1976 in a child living in a farm house (*Nime et al.* 1976). Since then case reports of immunocompetent, infected persons have suggested zoonotic origin of the infection because most patients have had close contact to animals, particularly calves with cryptosporidiosis (*Reese et al.* 1982, *Anderson et al.* 1982, *Current et al.* 1983, *Rahaman et al.* 1984, *Pitlik et al.* 1983, *Pohjola et al.* in print). Epidemiological surveys of rural populations particularly among persons occupationally exposed to cattle to evaluate the zoonotic implication has not until now been done.

In humans in our study the occurrence of cryptosporidiosis seemed to be positively associated with the duration of the daily exposure to cattle. 14.3 % of the persons occupationally exposed to cattle excreted oocysts compared to 2.6 % of their family members. From Bangladesh *Rahaman et al.* (1984) reported 7.9 % of 88 diarrheal animal attendants to excrete oocysts, additional positive cases being associated with 2 Cryptosporidium positive animal attendants.

Infections in animal handlers in our study may have resulted from the direct contact to cattle whereas in family members human-to-human transmission seems probable. This mode of transmission has been reported as case of possible hospital cross infection (*Baxby et al.* 1983) and as an accidental infection of a researcher while inoculating a rabbit for experimental purposes (*Blagburn et al.* 1983). In both reports, however, shedding of oocysts had been accompanied with diarrhea (*Baxby et al.* 1983, *Blagburn et al.* 1983).

Epidemiological knowledge of transmission chains of cryptosporidiosis on farms is unknown. Vertebrate hosts that might serve as sources of infection for calves include cats and mice, which both have been successfully infected with oocysts of calf origin in experimental work (*Current et al.* 1983, *Pavlasek* 1983). Moreover, both species can often be found in cowsheds in Finland. Feline as well as murine cryptosporidiosis usually occurs without clinical symptoms (*Current et al.* 1983, *Sherwood et al.* 1982). Besides these two species it is equally possible that human carriers are a source of infection for calves. As highly resistant — only formol saline and ammonia were found to be effective (*Campbell et al.* 1982) — the oocysts may keep their viability for long periods in the environment and transmit the infection through several chains of species.

Since cryptosporidiosis appears to be a calf-associated zoonosis and no specific therapeutic agent so far exists the infection can best be controlled by encouraging precautions in handling diarrheal calves and by insisting hygienic treatment of all fecal material.

In the light of present knowledge the sources of human cryptosporidiosis are the following: diarrheal newborn calves (*Reese et al.* 1982, *Anderson et al.* 1982, *Current et al.* 1983, *Rahaman et al.* 1984, *Pohjola et al.* in print), person-to-person transmission among children (*Hunt et al.* 1984, *Casemore & Jockson* 1984) and traveling to endemic areas (*Jokipii et al.* 1983, *Jokipii et al.* 1985). It seems that symptomless cryptosporidiosis may be frequent among animal handlers, whereas children and travelers develop gastroenteritis.

REFERENCES

Allen, A. V. H. & D. S. Ridley: Further observations on the formol-ether concentration technique for faecal parasites. J. clin. Pathol. 1970, *23*, 545—546.

Anderson, B. C.: Patterns of shedding of cryptosporidial oocysts in Idaho calves. J. Amer. vet. med. Ass. 1981, *178*, 982—984.

Anderson, B. C., T. Donndelinger, R. M. Wilkins & J. Smith: Cryptosporidiosis in a veterinary student. J. Amer. vet. med. Ass. 1982, *180*, 408—409.

Anderson, B. C. & R. F. Hall: Cryptosporidial infection in Idaho dairy calves. J. Amer. vet. med. Ass. 1982, *181*, 484—485.

Baxby, D., C. A. Hart & C. Taylor: Human cryptosporidiosns: a possible case of hospital cross infection. Brit. med. J. 1983, *287*, 1760—1761.

Blagburn, B. L. & W. L. Current: Accidental infection of a researcher with human Cryptosporidium. J. infect. Dis. 1983, *148*, 772—773.

Campbell, I., S. Tzipori, G. Hutchison & K. W. Angus: Effect of disinfectants on survival of Cryptosporidium oocysts. Vet. Rec. 1982, *111*, 414—415.

Casemore, D. P. & F. B. Jackson: Hypothesis: cryptosporidiosis in human beings is not primarily a zoonosis. J. Infect. 1984, *9*, 153—156.

Current, W. L., N. C. Reese, J. V. Ernst, W. S. Bailey, M. B. Heyman & W. M. Weinstein: Human cryptosporidiosis in immunocompetent and immunodeficient persons. Studies of an outbreak and experimental transmission. N. Engl. J. Med. 1983, *308*, 1252—1257.

Garcia, L., D. A. Bruckner, T. C. Brewer & R. Y. Schimizu: Techniques for recovery and identification of Cryptosporidium oocysts from stool specimens. J. clin. Microbiol. 1983, *18*, 185—190.

Henriksen, S. A. & J. F. L. Pohlenz: Staining of cryptosporidia by a modified Ziehl-Neelsen technique. Acta vet. scand. 1981, *22*, 594—596.

Hunt, D. A., R. Shannon, S. R. Palmer & A. E. Jephcott: Cryptosporidiosis in an urban community. Brit. med. J. 1984, *289*, 814—816.

Jokipii, L. S. Pohjola & A. M. M. Jokipii: Cryptosporidium: a frequent finding in patients with gastrointestinal symptoms. Lancet 1983, *ii*, 358—361.

Jokipii, L. S. Pohjola & A. M. M. Jokipii: Cryptosporidiosis associated with traveling and giardiasis. Gastroenterology 1985, *89*, 838—842.

Lasser, K. H., K. J. Lewin & F. W. Ryning: Cryptosporidial enteritis in a patient with congenital hypogammaglobulinemia. Hum. Pathol. 1979, *10*, 234—240.

Levine, N. D.: Taxonomy and review of the coccidian genus. Cryptosporidium (Protozoa, Apicomplexa). J. Protozool. 1984, *31*, 94—98.

Meisel, J. L., D. R. Perera, C. Meligro & C. E. Rubin: Overwhelming watery diarrhea associated with a Cryptosporidium in an immunosuppressed patient. Gastroenterology 1976, *70*, 1156—1160.

Miller, R. A., R. E. Holmberg & C. R. Clausen: Life-threatening diarrhea caused by Cryptosporidium in a child undergoing therapy for acute lymphocytic leucemia. J. Pediat. 1983, *103*, 256—259.

Nime, F. A., J. D. Burek, D. L. Page, M. A. Holscher & J. H. Yardley: Acute enterocolitis in a human being infected with the protozoan Cryptosporidium. Gastroenterology 1976, *70*, 593—598.

Pavlasek, I.: Experimental infection of cat and chicken with Cryptosporidium sp. oocysts isolated from a calf. Folia Parasitol. 1983, *30*, 121—122.

Pitlik, S. D., V. Fainstein, D. Garza, L. Guarda, R. Bolivar, A. Rios, R. L. Hopfer & P. A. Mansell: Human cryptosporidiosis: Spectrum of Disease. Report of six cases and review of literature. Arch. Intern. Med. 1983, *143*, 2269—2275.

Pohjola, S.: Cryptosporidiosis, a literature review. Suom. Eläinlääk. L. 1983, *89*, 146—153.

Pohjola, S. H. Oksanen, E. Neuvonen, P. Veijalainen & K. Henriksson: Certain enteropathogens in calves of Finnish dairy herds with recurrent outbreaks of diarrhea. Prev. Vet. Med. 1986, *3*, 547—558.

Pohjola, S. H. Oksanen, L. Jokipii & A. M. M. Jokipii: Outbreak of cryptosporidiosis among veterinary students. Scand. J. infect. Dis., in print.

Pohlenz, J., H. W. Moon, N. F. Cheville & W. J. Bembrick: Cryptosporidiosis as a probable factor in neonatal diarrhea of calves. J. Amer. vet. med. Ass. 1978, *172*, 452—457.

Rahaman, A. S. M. H., S. C. Sanyal, K. A. Al-Mahmud, A. Sobhan, K. S. Hossain & B. C. Anderson: Cryptosporidiosis in calves and their handlers in Bangladesh. Lancet 1984, *ii*, 221.

Reese, N. C., W. Current, J. V. Ernst & W. S. Bailey: Cryptosporidiosis of man and calf: a case report and results of experimental infections in mice and rats. Amer. J. trop. Med. Hyg. 1982, *31*, 226—229.

Ritchie, L. S.: An ether sedimentation technique for routine stoll examinations. Bull. US. Army Dept. 1948, *8*, 326.

Sherwood, D. K. W. Angus, D. R. Snodgrass & S. Tzipori: Experimental cryptosporidiosis in laboratory mice. Infect. Immun. 1982, *38*, 471—475.

Sloper, K. S., R. R. Dourmashkin, R. B. Bird, G. Slavin, & A. D. B. Webster: Chronic malabsorption due to Cryptosporidium in a child with immunoglobulin deficiency. Gut 1982, *23*, 80—82.

Tzipori, S., I. Campbell, D. Sherwood & D. R. Snodgrass: An outbreak of calf diarrhea attributed to cryptosporidial infection. Vet. Rec. 1980, *107*, 579—580.

Tzipori, S.: Cryptosporidiosis in animals and humans. Microbiol. Rev. 1983 a, *47*, 84—96.

Tzipori, S., M. Smith, C. Birch, G. Barnes & R. Bishop: Cryptosporidiosis in hospital patients with gastroenteritis. Amer. J. trop. Med. Hyg. 1983 b, *32*, 931—934.

Tyzzer, E. G.: A sporozoan found in the peptic glands of the common mouse. Proc. Soc. exp. Biol. Med. 1907, *5*, 12—13.

Weisburger, W. R., D. F. Hutcheon, J. H. Yardley, J. C. Roche, W. D. Hillis & P. Charoche: Cryptosporidiosis in an immunosuppressed renal transplant recipient with IgA deficiency. Amer. J. clin. Pathol. 1979, *72*, 473—478.

Zar, J. H.: Biostatistical Analysis. Prentice Hall Inc, Englewood Cliffs, New Jersey 1974.

SAMMANDRAG

Sporadisk Cryptosporidios hos en landsortsbefolkning är asymptomatisk och förbunden med kontakt med nötkreatur.

Avföringsprov från 233 personer representerande 80 hushåll undersöktes. Försökspersonerna fyllde också i ett frågeformulär. Med hjälp av formalin-eter koncentrering i kombination med acid-fast färgning hittades Cryptosporidium oocystor hos 7 (3 %) personer. 63 personer var i kontakt med nötkreatur, och asymptomatisk cryptosporidios diagnostiserades hos 6 av dem. Cryptosporidios konstaterades inte hos en enda av de 51 personer som var i kontakt med andra djur än nötkreatur, och ingen av de 7 Cryptosporidium positiva personerna hade rest utomlands. Vi tog också avföringsprov från 15 kalvar från 7 gårdar. 6 kalvar (40 %) hade cryptosporidier och manifest enterit påvisades hos de kalvar som var under 1 månad gamla. 5 av 14 gårdar hade problem med neonatal kalvdiarre. Human cryptosporidios var vanligare på sådana problemgårdar. Resultaten visar att human cryptosporidios hos en landsortsbefolkning i huvudsak är asymptomatisk och främst förekommer hos invånade på gårdar med nötkreatur.

(Received December 4, 1985).

Reprints may be requested from: Suvi Pohjola, the Department of Food and Environmental Hygiene, College of Veterinary Medicine, P. O. Box 6, SF-00551 Helsinki, Finland.